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Antibacterial Activity of Silver, Gold and Amoxicillin-Silver Nanoparticles against Methicillin Resistant *Staphylococcus aureus* - an invitro Study

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ABSTRACT

Introduction: Methicillin-resistant *staphylococcus aureus*, a multi drug resistant bacterium is responsible for a broad spectrum of human and animal diseases ranging from skin infections to severe disorders as pneumonia, endocarditis and septicemia causing morbidity and mortality worldwide. This has led to growing interest in exploring novel methods to treat increasing drug resistance.

Aim/Objectives: Aim of our study was to determine the anti-MRSA activity of silver and gold nanoparticles alone, also combination of amoxicillin and silver nanoparticles combination.

Materials and Methods: The antimicrobial activity of silver and gold nanoparticles was evaluated using Disc diffusion method and amoxicillin in combination with silver nanoparticles by determining the colony forming units.

Results: Silver nanoparticles showed anti-MRSA activity whereas gold nanoparticles, amoxicillin and silver nanoparticles combination showed no activity against MRSA clinical isolates.

Key Words: Methicillin Resistant *Staphylococcus aureus*, Silver Nanoparticles, Gold Nanoparticles, Amoxicillin, Antibiotic Susceptibility Testing, Multi-Drug Resistance

INTRODUCTION

Staphylococcus aureus is one of the most infamous and widespread bacterial pathogens, causing a hard to estimate number of uncomplicated skin infections and probably hundreds of thousands to millions of more severe, invasive infections globally per year.[1] This bacterium is found in the normal skin microbiota of both animals and humans of the healthy human population[2] causing abscesses, lung infections, bacteremia, endocarditis and osteomyelitis.[3] *S. aureus* infections are particularly problematic due to frequently occurring antibiotic resistance in *S. aureus* isolates, among which Methicillin-Resistant *Staphylococcus aureus* (MRSA) are the most important clinically.[4] Infections by MRSA are accompanied by increased mortality, morbidity, and hospital stay, as compared to those caused by Methicillin Sensitive *Staphylococcus aureus* (MSSA).[5] MRSA tend to be multi-drug resistant, that is resistant not only to β-lactam antibiotics but also to a range of different antibiotic classes, such as fluoroquinolones, tetracyclines, macrolides, lincosamides and aminoglycosides.[6] In previous years, strains have emerged with an intermediate susceptibility or full resistance to vancomycin (Vancomycin Intermediate *Staphylococcus aureus* (VISA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA), respectively), the antibiotic that has represented the cornerstone of therapy for MRSA for two decades.[7]

The increase in antibiotic resistance led to an increase in research to find alternative agents and treatments, including the use of Nanoparticles (NPs). Several characteristics of NPs make them alternatives to traditional antibiotics. First, the large surface-area-to volume ratio of NPs increases the contact area with target organisms. NPs can act as nanoscale molecules interacting with bacterial cells, regulating cell membrane penetration, and interfering with molecular pathways.[8,9]

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Silver nanoparticles (SNPs) are one of the most important NPs that play significant roles in biomedical applications such as wound healing, cell imaging, diagnosis, disease treatment, and contraceptive devices.[10] The key mechanisms of action of silver nanoparticles are the release of silver ions which enhances antimicrobial activity[11], cell membrane disruption, and DNA damage.[9] Gold nanoparticles have generated considerable interest within the biomedical community for *in vivo* imaging, drug delivery, and thermotherapy for the localized killing of cancer cells.[12] Utilizing combination therapy to battle bacterial infection is also one of the proposed approaches to treat infections with antibiotic resistant bacterial strains.[13]

The objective of the current research was to determine the activity of silver and gold nanoparticle alone and also silver nanoparticle in combination with amoxicillin. The relevance of the concentration/dosage and the time to the antibacterial activity were determined by estimating zone of inhibition and colony forming units.

MATERIALS AND METHODS

The present study was carried out at Department of Medical Microbiology, School of Medical Education during the period of June to August (2022). 30 clinical isolates of MRSA strains were collected from various diagnostic labs in Kerala. Clinical isolates were reconfirmed as MRSA by gram staining, yellow colored colonies on Mannitol Salt Agar (MSA) and based on susceptibility to Cefoxitin (30µg) as prescribed by CLSI (M100-S32).[14]Antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method was also done for Penicillin (10 Units), Erythromycin (15µg), Clindamycin (2µg), Gentamicin (10µg), Mupirocin (200 µg-for detection of high level resistance), Linezolid (30µg), Quinupristin/Dalfopristin (12µg), Linezolid (30 µg), Tetracycline (10 µg), Ciprofloxacin (5 µg), Trimethoprim Sulfamethoxazole (1.25/23.75 µg) as prescribed CLSI Standards (M02-A13).[15] Inducible resistance to clindamycin was tested by 'D test' as prescribed by CLSI (M100-S32).[14]

Silver (10nm) and gold (20 nm) nanoparticle colloidal solution were procured from Reinste Nanoventures Pvt. ltd, Noida. All other reagents culture media and antibiotic discs were obtained from HiMedia Laboratories Mumbai,India.

Determination of silver and gold nanoparticle activity

MRSA was inoculated into BHI broth and its turbidity will be adjusted to 0.5 McFarland standard before the performance of sensitivity. Lawn culture was done on MHA plates using isolated MRSA strains. Sterile discs were placed on it and different volumes of (10µl, 20µl, 40µl, 60µl, 80µl, 100µl) silver and gold nanoparticles was incorporated onto it.MHA plates are then incubated at 37˚C for 18-24 hours. The zone of inhibition was measured to the nearest in mm after incubation. All test was done in triplicates and mean diameter was calculated.

Determination of amoxicillin and silver nanoparticles activity

MRSA was grown overnight in BHI broth at 37°C.After incubation, the broth culture was centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded and the cells were washed twice with sterile distilled water to remove any traces of protein left over from media. The cells were then resuspended in sterile distilled water and its density is adjusted to 0.5 McFarland standard. In a 96-well microtitre plate, 50µl of cell suspension was mixed with 50µl of Amoxicillin and varying concentrations (10 µg, 20 µg, 40µg, 80 µg, 160 µg) of silver nanoparticles suspensions. The final volume was adjusted to 200µl and the plates was incubated at room temperature without shaking for 1 hour.Aliquots of 10 µl were taken out at 0, 30 and 60 minutes and plated on BHI agar using a L-spreader.

STATISTICAL ANALYSIS

The study was approved by the institutional ethical committee at School of Medical Education. The data was analyzed using Microsoft excel 2019 and statistical package for the social sciences (SPSS-18).

RESULTS

Antibiotic susceptibility pattern of MRSA

The antibiotic susceptibility pattern of MRSA isolates obtained in the present study is given in figure 1. Out of 30 clinical samples, 100% (n=30) were resistant to cefoxitin. 20% of isolates were sensitive to erythromycin while 3.33% isolates were of intermediate susceptibility. The remaining 76.66% were resistant. Clindamycin resistance was observed in 33.33% of the isolates, 43.33% were sensitive to clindamycin, and the remaining 23.33% were of intermediate susceptibility. Ciprofloxacin resistance was observed in 43.33% of isolates and 23.33% were classified as intermediate. 43.33% of isolates were sensitive to ciprofloxacin. 96.66% MRSA isolates were sensitive to linezolid while 3.33% exhibited resistance. 80% displayed sensitivity,16.66% were resistant and 3.33% was classified as intermediate susceptibility to Trimethoprim-sulfamethoxazole. 100% isolates were sensitive to mupirocin. Quinupristin/Dalfopristin resistance was observed in 80% of the isolates while 20% were sensitive. 40% of isolates were sensitive to tetracycline while 13.33% were resistant and the remaining 46.66% were of intermediate susceptibility. 46.66% displayed sensitivity 40% were resistant and 13.33%

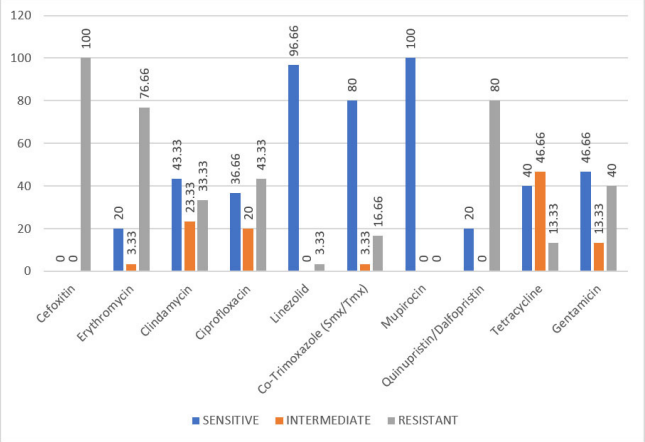

Figure 1: Antibiotic sensitivity pattern of MRSA isolates

was classified as of intermediate susceptibility to gentamicin. Out of 30 clinical isolates, 37% tested positive for inducible clindamycin resistance, while rest of the 68% isolates were negative for D -test.

Activity of silver nanoparticles against MRSA isolates

The result showed that zone of inhibition increases with increase in concentration of silver nanoparticles as shown in fig 2.

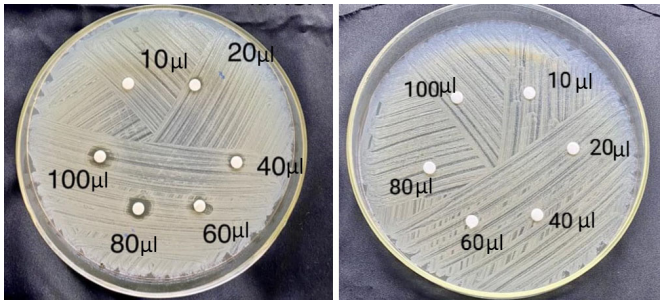

Figure 2: showing silver and gold nanoparticle activity against MRSA isolates.

Activity of gold nanoparticles against MRSA isolates

The result showed that gold nanoparticles exhibited no activity against all the 30 isolates of MRSA as shown in fig 2.

Comparative activity of silver and gold nanoparticles against MRSA (n=30)

Silver nanoparticles showed increase in zone of inhibition with increase in volume but gold nanoparticles did not have any activity as volume increased as shown in fig 3. The comparative activity of gold and silver nanoparticle against MRSA isolates was analyzed using ANOVA-one way method and was found not significant, (P value significant if<0.05).

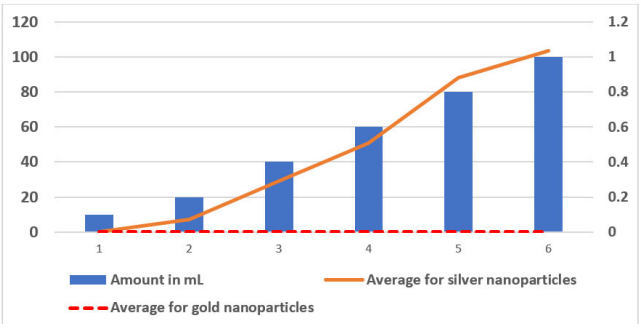

Figure 3: Showing comparative activity of gold and silver nanoparticles against MRSA isolates.

Activity of Silver nanoparticles with amoxicillin against MRSA isolates

Amoxicillin in combination with varying concentrations of silver nanoparticles showed no activity against MRSA.

DISCUSSION

Infections are a dominant contributor to global disease burden. infections caused by multi-drug resistant organisms like MRSA are emerging as causes of morbidity and mortality worldwide. The treatment of infectious diseases is hampered by drug resistance, indicating that there is a serious need to develop new therapeutics which can overcome drug resistance. Therapeutic agents such as antibody-based therapeutics, metal-based nanoparticles, etc. have been developed for the treatment of infectious diseases.[16] NanoParticles exhibiting antibacterial activities can target multiple biomolecules and have the potential to reduce or eliminate the evolution of MDROs. However, the translation of NPs to clinical use requires in vitro and in vivo studies.so the current study aims to evaluate the *invitro* anti MRSA activity of silver and gold nanoparticle and also silver nanoparticles in combination with amoxicillin.

In the present study, silver nanoparticles exhibited activity against MRSA clinical isolates which is comparable to study of Ansari et al. in their study also anti-MRSA activity increased with increasing concentration of silver nanoparticles as exhibited in our study.[17] Gold nanoparticles did not exhibit anti-MRSA activity in the current study which is in contrast to the study of li X et al. reported anti-MRSA activity for gold nanoparticles, the difference in activity might be due to the 2 nm diameter of gold nanoparticle used by Li X et al. to the 20 nm gold nanoparticle used in this study.[18]

Whereas combination of silver nanoparticles and Amoxicillin showed no activity against MRSA which is contrary to other study of Augmented antibacterial activity of ampicillin with silver nanoparticles against MRSA by Surwade et al. that states the combination of silver nanoparticles and a

low concentration of ampicillin has potential in treating infections with drug resistant bacteria, at higher concentration, ampicillin completely coats the silver nanoparticle to form a corona. This corona provides a protective layer against direct interaction with the MRSA cell surface, hence decreasing antimicrobial activity,[19] which might be the reason amoxicillin with silver nanoparticles didn't exhibit anti-MRSA activity.

CONCLUSION

Silver nanoparticles exhibited anti-MRSA activity while gold nanoparticles didn't. Amoxicillin combined with silver nanoparticles couldn't revert resistance to amoxicillin. We recommend usage of nanoparticles with a smaller diameter in future studies. As conventional antibiotics with typical mechanisms of actions are gradually getting resistant by MRSA at a faster pace researchers should pursue to develop alternative approaches to the affective treatment of resistant bacterial infections.

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Ethical approval -The study was approved by Institutional Ethical Committee, Ref No: IEC/17/MICRO/SME-GNR/2021.

Authors' Contribution: All authors have equally contributed to the present study.

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